

UNIVERSITY OF MISSOURI.

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IN AGRICULTURAL CHEMISTRY  
PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS  
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## THE PLURAL NATURE OF VITAMIN B.

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Ever since the existence of vitamin B has been generally accepted, suggestions have appeared from time to time that this dietary factor is in reality a mixture, and not a single substance. The earlier literature on this topic has been summarized repeatedly, and citation of any but the more recent papers seems unnecessary at this time. The evidence has become continually more convincing, but the papers of Smith and Hendrick (1) and of Goldberger and collaborators (2) are the first that carried conviction to the greater body of readers. Smith and Hendrick examined two substances for vitamin B activity, a vitamin B pierate and autoclaved yeast. Neither alone was adequate as a source of vitamin B, but a combination of the two was fairly satisfactory. Goldberger and associates presented a greater body of evidence than Smith and Hendrick, but used essentially the same method. They observed that large quantities of either an alcohol extract of maize meal or a preparation of autoclaved yeast are inadequate as a source of vitamin B, but a combination of the two is effective.

Salmon (3) prepared similar factors from the leaves and seeds of the velvet bean and other plants, but by the use of fullers' earth obtained the active agents in a more concentrated form. In a later paper (4), these findings have been elaborated somewhat. A careful study of this topic has also been reported by Chick and Roscoe (5) who used methods similar to those just described. Their evidence also seems to indicate conclusively that vitamin B is a mixture. A number of other workers have made similar reports (6), in entire agreement with those just cited, but most of them are less elaborate and will not be described here in detail. It is probably unnecessary to point out that when a separation of the vitamin components has been attained, one is chiefly antineuritic, the other growth-promoting. Autoclaved preparations retain the latter factor, but are completely devoid of the former.

We believe then that vitamin B does contain at least two factors. It seems to us, however, that the evidence would be even more conclusive if a single preparation were used, in itself a complete source of vitamin B, but which with suitable manipula-

tion manifests on the one hand only antineuritic activity, on the other only growth-promoting activity. A mixture of these two modified preparations, however, should be a satisfactory source of vitamin B. The possibility of obtaining such preparations seemed realized when we observed (7) that a vitamin B preparation exposed to a quartz mercury arc retains its antineuritic activity, but that it loses some other essential property. Irradiated yeast or an irradiated vitamin B concentrate was effective in relieving pigeons from an acute attack of polyneuritis, but final collapse, due to the absence of some other essential, was the inevitable sequel. Autoclaved yeast, it will be recalled, loses its antineuritic potency, but as shown by other workers, retains growth-promoting activity. These facts suggest the possibility that vitamin B is a mixture of two components, and that one is destroyed by irradiation, the other by high temperatures. We decided, therefore, to study in more detail each of these modified preparations, and also to determine whether there is any supplementary relation between them.

As experimental animals, rats, chicks, and pigeons were used, and usually they were placed under observation at the earliest practicable age. In some series a few of the rats were only 21 days of age when the initial weights were taken, in others they were as much as 28 days old. In each series, however, the rats were, so far as possible, equally distributed between the various groups, according to age, sex, weight, and parentage. The chicks were newly hatched in every case, and the pigeons varied from 6 to 8 weeks in age by the time they had learned to consume readily our experimental diets. We were not able in every case to secure a sufficient number of pigeons of the same variety, but most of them were Silver Kings. A number of White Kings were also used, and a few Yellow Kings. In our experience the Silver Kings are hardiest and most suitable for this type of work.

With one exception the same ration, No. 465, was fed to all experimental animals. This diet is regarded as practically free from vitamin B, and has the following composition:

|             | <i>per cent</i> |               | <i>per cent</i> |
|-------------|-----------------|---------------|-----------------|
| Casein*     | 20              | Cod liver oil | 5               |
| Corn-starch | 58              | Cellulose     | 3               |
| Lard        | 10              | Salts (8)     | 4               |

\* Washed continuously for 1 week with dilute acetic acid.



One group of chicks, however, received its vitamin B supplement incorporated in the ration. The diet was identical with Ration 465, except that 15 parts of yeast had been added and an equal quantity of starch removed. This yeast component was, however, modified in various ways. Thus some chicks received untreated yeast, some a heated, some an irradiated preparation, while others received a mixture of equal parts of the two modifications.

Usually the food was kept before the animals continually to be consumed at will, but in one trial the pigeons were hand-fed to insure an equal intake of food by all groups. In order to facilitate the process of hand feeding, the various constituents of the ration, except the cod liver oil, were mixed, moistened, and after being moulded into small pellets, dried. These pellets were then coated evenly with the cod liver oil and kept in a cold room until used. The vitamin B supplements were always fed separately from the rest of the ration, except in the one instance just mentioned of the chicks. The daily portions of the supplement for the birds were placed in gelatin capsules and were hand-fed. The supplements for the rats were placed in glass dishes, and in practically every instance they were consumed immediately.

Two sources of vitamin B were used, Harris dried yeast, and a vitamin B concentrate prepared by ourselves from fresh brewers' yeast,<sup>1</sup> by the method of Osborne and Wakeman (9). Our experience with commercial concentrates has not been satisfactory, and we regard negative results obtained with them as of doubtful significance.

Prior to irradiation the material was ground to a fine powder and scattered in a thin layer, not over 0.5 mm. thick, on a glass plate 25 × 30 cm. The plate was directly below the quartz mercury arc,<sup>2</sup> at a distance of 25 cm., and each preparation was exposed for a period of 10 hours. We are unable to state quantitatively the amount of radiant energy per unit of area of the exposed material, but the lamp gives a brilliant spectrum ex-

<sup>1</sup> We take this opportunity to express our obligation to Dr. O. F. Steidemmann, Chief Chemist of the Anheuser Busch Company, of St. Louis, who kindly placed at our disposal several hundred pounds of brewers' yeast.

<sup>2</sup> The arc is manufactured by the Cooper Hewitt Electric Company, and operates at 117 volts and 3.8 amperes.

tending far into the ultra-violet region. The temperature of the irradiated materials varies somewhat, depending on the temperature of the room, but the highest reading observed was 40.1°.

The preparations to be heated were thoroughly moistened with water, heated for 2 hours in an autoclave at 120°, then dried and powdered.

In practically every series of observations, the animals were divided into four groups, corresponding to the four modifications of the vitamin that were used. The first group received the autoclaved supplement, and the second received the irradiated material. Each member of Group 3 received one-half as much of the autoclaved supplement as did the individuals of Group 1, and in addition one-half as much of the irradiated supplement as did the individuals of Group 2. Group 4 received the untreated preparation. The total amount of supplement supplied to the individuals of the various groups was the same.

The rats were confined in small individual cages, made of hardware cloth, with raised screen bottoms. The pigeons and chicks were confined by the group method. Since the chicks were placed on the experimental rations immediately after hatching, they were kept in electrically heated brooders until 4 weeks of age, to assure a suitable temperature during that critical period.

In our first trials yeast itself was used, and though the results were decisive, we believe the vitamin concentrate is much more suitable for experimental purposes. Some of our observations with yeast are presented in Fig. 1.

It will be noted that the mortality on both of the modifications, either heated or irradiated yeast, was 100 per cent. The survival period on the irradiated yeast was a little longer than on the autoclaved material, but we attach no significance to that, other than indicating that the reaction due to exposure may not have gone to completion.

Our observations on chicks were less satisfactory, due chiefly we believe, to the fact that their nutritional requirements (10) are partially unknown as yet. Occasionally a group will grow at an almost normal rate on a synthetic ration, but that is rare, and the variability is always great. We have, therefore, selected a group in which the controls made satisfactory growth, and have incorporated the data in Fig. 2.



It will be noted that the chicks receiving the mixture of irradiated and autoclaved yeast did as well as those receiving untreated yeast, and much better than those receiving either the irradiated or autoclaved material alone.

From our standpoint the behavior of the chicks that received autoclaved yeast was very interesting. Two of them did as well as chicks usually do on untreated yeast, and they were in good condition when removed from observation at 9 weeks.

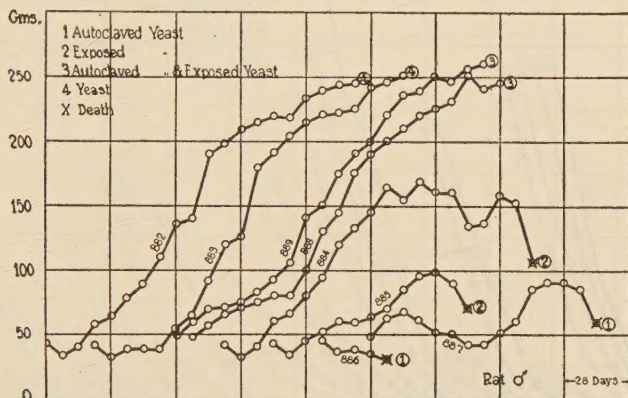


FIG. 1. This chart shows that for rats neither autoclaved yeast nor irradiated yeast (Groups 1 and 2) is an adequate source of vitamin B. The mixture (Group 3) of these preparations is, however, not much less effective than the untreated material (Group 4). The supplements were fed at the following levels daily: from the 7th day, 0.2 gm.; from the 21st day, 0.4 gm.; from the 28th day, 0.5 gm.; from the 42nd day, 0.6 gm.; from the 56th day, 0.75 gm.; from the 70th day, 1 gm.; from the 105th day, 1.25 gm.

In the next series rats were employed, and the yeast concentrate was given, as shown in Fig. 3. The response was much more prompt than when yeast was used. The rats receiving the autoclaved concentrate lost weight almost from the beginning and succumbed within 5 weeks at the latest. The loss of weight of those receiving the irradiated fraction was less marked, but the final outcome was the same. In contrast with these, however, the rats receiving the mixture of autoclaved and irradiated fractions did practically as well as those that received the untreated fraction.

Lastly we are presenting in Figs. 4 and 5 the results obtained with some of the pigeons that received the vitamin concentrates. Those in Fig. 4 were fed *ad libitum*, and those in Fig. 5 all received the same amount of food per unit of weight.

It will be observed in both instances that all pigeons receiving either the irradiated or autoclaved concentrate lost weight and succumbed shortly, none surviving longer than 7 weeks. Those

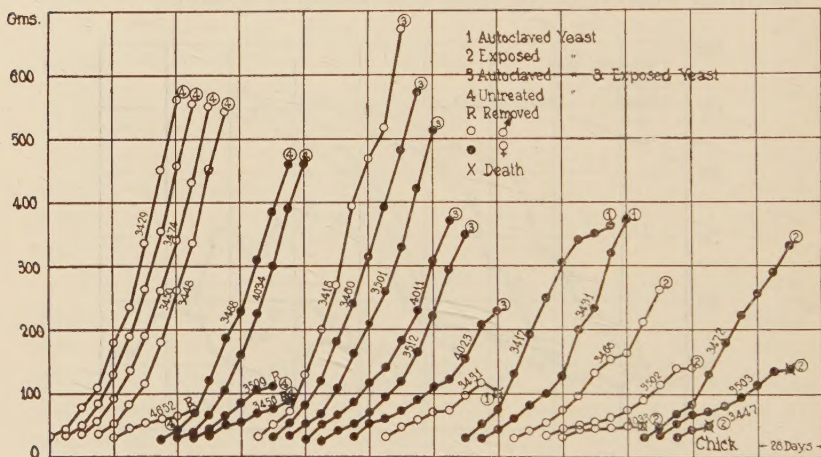


FIG. 2. The chicks receiving the mixture (Group 3) of treated yeast supplements did as well as those that received the untreated material (Group 4). Either of these preparations is apparently more suitable than is either the autoclaved (Group 1) or the irradiated material (Group 2). In this series the vitamin B supplements constituted 15 per cent of the ration. Originally there were seven chicks in each of Groups 1, 2, and 3. We have omitted, however, all those that died within the first 2 weeks.

receiving the mixture of the autoclaved and irradiated fractions, however, did as well as those receiving the untreated supplement.

It will be noted that the groups receiving either the untreated material or the mixture, shown in Fig. 4, either maintained their weight or made some increase. Similar groups in Fig. 5 were on a restricted food intake, and these at first suffered large losses in weight and then seemed to come to equilibrium. The continued rapid loss of weight in the groups receiving singly the autoclaved or irradiated preparations (Fig. 5) was unexpected, however, for



owing to hand feeding they were receiving more food at the last than similar birds shown in Fig. 4 would consume voluntarily. Inspection of the cages yielded only slight evidence of regurgitation, though we cannot be positive that it did not occur. In similar observations Marrian, Baker, Drummond, and Woollard (11) estimate that not over 20 per cent of the forcibly fed food was regurgitated. Their birds, however, suffered little if any loss in weight. These observers also reported spontaneous cures from polyneuritis, but we did not note such cases. Temporary relief

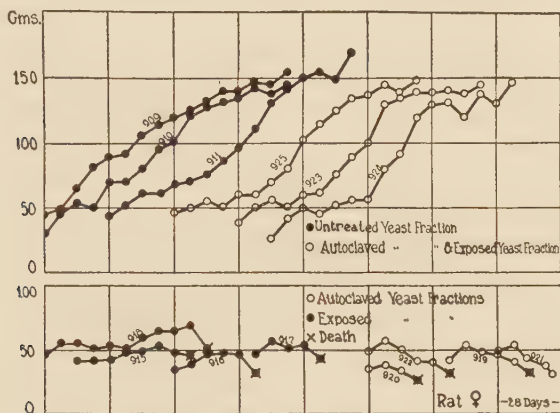


FIG. 3. All animals in this series received the vitamin B concentrate. The mixture of irradiated and autoclaved fractions was little if any less effective than the untreated material. When fed alone neither the autoclaved supplement nor the irradiated supplement was adequate as a source of vitamin B. For the first 21 days, all animals received 50 mg. of the concentrate, and then the amount was increased to 80 mg. This was increased to 150 mg. on the 42nd day, and to 200 mg. on the 70th day.

from acute attacks was frequently observed, but there were no real recoveries. Possibly this difference in behavior may be explained by the immaturity of the pigeons we were using.

Since we believe the deaths of the pigeons receiving autoclaved and irradiated vitamin B supplements were due to different inadequacies, the symptoms preceding collapse are of some interest. Until shortly before death there were no perceptible differences in their behavior. They became apathetic, and were in an obviously poor nutritional condition, but at the end the

majority of those receiving the autoclaved preparations developed symptoms of polyneuritis, with a typical head retraction. Many, however, did not display characteristic symptoms of any kind. Of those receiving the irradiated fraction, a few gave some indication of head retraction, but practically all of them passed into a stupor which soon ended in death.

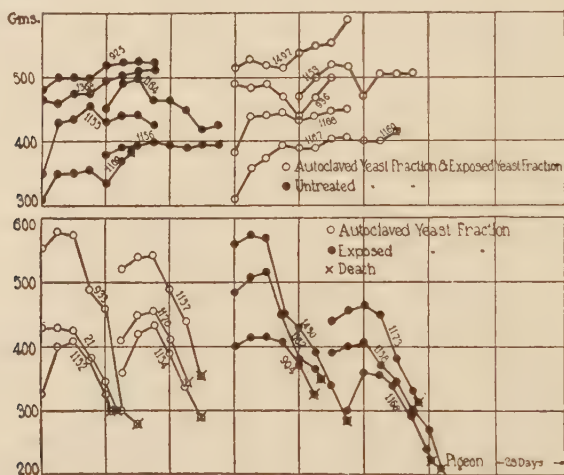


FIG. 4. This chart shows the effect of high temperature and of exposure to ultra-violet rays, on the concentrate when used as a source of vitamin B for pigeons. The mixture of these concentrates was apparently as active as the untreated material. When fed alone neither the autoclaved supplement nor the irradiated supplement was adequate as a source of vitamin B. For the first 8 days all the pigeons received an adequate ration of natural foodstuffs, but powdered finely so as to resemble our synthetic rations. On the 9th day they were given Ration 465, and 200 mg. daily of the supplements as described above. On the 23rd day the amount of supplement was increased to 300 mg. daily. The concentrate was fed separately, by hand, in all cases.

In order to show more clearly the supplementary relation between the irradiated and autoclaved vitamin B carriers, we are presenting in Table I a brief summary of our data. We are omitting, however, the series of chicks (Fig. 2) which received yeast incorporated in the ration as a source of this supplement, since our observations were discontinued before any evidence was obtained as to the length of the survival period.



Table I shows that when either irradiated or autoclaved materials are offered as the sole source of vitamin B, there are no survivors. However, the mixture of supplements was adequate, although the percentage of survivors was not always 100. Since, however, the different species do not behave alike in feeding trials, we shall consider them separately. Rats are much the

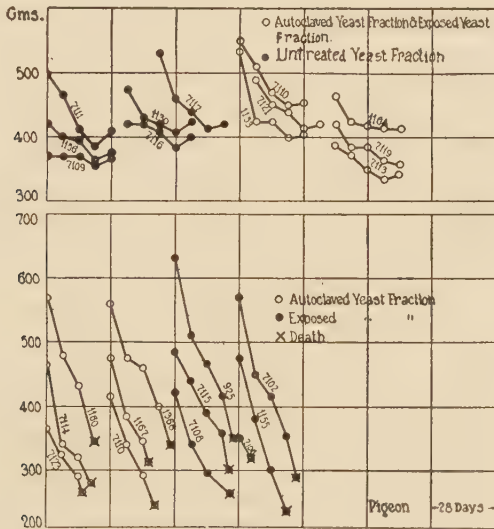


FIG. 5. The results shown in this chart are in entire agreement with those given in Fig. 4. The squabs shown in Fig. 4 were fed *ad libitum*, while those described in Fig. 5 were hand-fed, all receiving the same amount of food per unit of weight. For the first 14 days each individual received daily 1 gm. of food for each 20 gm. of body weight. On the 15th day this quantity was changed to 1 gm. of food for each 30 gm. of body weight. The amount of supplement given was unchanged, 200 mg. daily for each individual.

simpler to handle, chiefly because their nutritional requirements are better understood and because they consume readily almost any adequate and suitable diet. Squabs require some training before they will eat at all, and our experience is that they require a second training before they will eat the finely powdered synthetic rations we employ. We did not appreciate this fact until the first experiment was under way, and we believe most of this group

would have starved on rations of this type, even if the diets were entirely adequate. In later trials the birds were all given an adequate ration, similar in appearance to our experimental diets, until their weights made it clear they had learned to eat. Many were hand-fed until they were satisfactorily trained, to prevent voluntary starvation. Feeding trials of this nature with chicks are uncertain at present, owing to the limitations of our knowledge of their nutritional requirements, and the variability within groups is always perplexing.

TABLE I.  
*Effect of Heat and of Exposure to Ultra-Violet Rays, on Vitamin B  
Component of Diet.*

| Supplement used.*        | Experimental animal. | No. of animals. | Percentage of survivors. |
|--------------------------|----------------------|-----------------|--------------------------|
| Irradiated.              | Rat.                 | 15              | 0                        |
|                          | Pigeon.              | 27              | 0                        |
|                          | Chick.               | 13              | 0                        |
| Autoclaved.              | Rat.                 | 15              | 0                        |
|                          | Pigeon.              | 27              | 0                        |
|                          | Chick.               | 13              | 0                        |
| Irradiated + autoclaved. | Rat.                 | 14              | 100                      |
|                          | Pigeon.              | 27              | 70                       |
|                          | Chick.               | 12              | 42                       |
| Untreated.               | Rat.                 | 9               | 89                       |
|                          | Pigeon.              | 21              | 76                       |
|                          | Chick.               | 13              | 61                       |

\* This includes both yeast and the Osborne-Wakeman concentrate.

To return then to the feeding trials with the mixture of supplements, it will be noted that it is at least a fairly adequate source of vitamin B. Of the rats on this diet, all were making some growth, and were in a satisfactory nutritional state when all their companions receiving the supplements separately had succumbed. In contrast to the rats, there were several mortalities among the pigeons and chicks receiving the mixture of vitamin concentrates. The percentages of survivors among the control groups were not much larger, however, and we do not regard the differences as necessarily significant.

A few observations were made on the body temperature of pigeons receiving, in some cases autoclaved, in others irradiated supplements. These records are scattered and limited in number, but they indicate distinct differences in physiological behavior. The body temperature of those receiving the autoclaved supplement was markedly lowered at least a week before the onset of characteristic symptoms. In one case a pigeon seemed to be in a low nutritional state and was found to have a body temperature of  $102.8^{\circ}$ . 7 days later, a few hours before the bird died, a reading of  $100.3^{\circ}$  was obtained. Another pigeon gave a reading of  $104.0^{\circ}$  8 days before death, and  $102.4^{\circ}$  on the last day it was observed alive. In a third case the temperature on the day preceding death was  $99.0^{\circ}$ . Similar reports on the effect of a deficiency of the antineuritic vitamin have been made by others (11, 12).

Apparently the effect of the irradiated supplement on the heat-regulating mechanism is quite different, as the lowest reading observed, taken the day preceding death, was  $104.4^{\circ}$ . Two other readings were taken the last few days before death, one of  $106.5^{\circ}$ , the other of  $107.6^{\circ}$ . The body temperature of pigeons receiving the mixture of supplements ranged from  $105.0$ – $108.5^{\circ}$ .

In view of the confusion that now exists in the terminology of vitamin B, a few comments may be pertinent. In the first place, we regard the designation "growth-promoting," so frequently applied to one of the vitamin B constituents, as unsuitable. Indirectly it does promote growth, by making possible a more normal nutritional condition, but in this sense, the antineuritic, the antiophthalmic, the antiscorbutic, and the antirachitic vitamins are also growth-promoting. Until we have some understanding of the chemical structure of the vitamins, or until we have more definite knowledge of their functions, a logical terminology is impossible. We believe, however, that at present the term "antidystrophic" is less objectionable, and less misleading, than is growth-promoting. From our point of view it seems desirable, for the present, to retain the nomenclature now generally in use, and to amplify it as new facts become available. We suggest, therefore, that in the future the term vitamin B should include only the antineuritic factor; and that the so called growth-promoting substance be designated as vitamin F.



## SUMMARY.

1. Under the experimental conditions we observed, vitamin B carriers become inadequate after exposure to ultra-violet rays for a period of 10 hours.

2. These irradiated materials retain antineuritic activity, but lose their so called growth-promoting properties.

3. Irradiated and autoclaved vitamin B preparations are both inadequate, when used as the sole source of the vitamin. There is a supplementary relation between them, however, and a mixture of the two is a potent source of vitamin B.

4. Vitamin B, as the term has been used in the past, is a mixture of at least two distinct vitamins.

5. It is suggested that the designation vitamin B be reserved for the antineuritic factor; the so called growth-promoting factor should be given an individual designation and be known as vitamin F.

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